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## Edema Disease-Like Brain Lesions in Gnotobiotic Piglets Infected with *Escherichia coli* Serotype O157:H7

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**Gnotobiotic piglets inoculated with *Escherichia coli* serotype O157:H7 strains that produced Shiga-like toxin II developed brain lesions similar to those observed in edema disease of swine, including arteriolar necrosis and malacia. Loss of ability to produce Shiga-like toxin II resulted in loss of ability to cause brain lesions.**

Enterohemorrhagic *Escherichia coli* (EHEC) of serotype O157:H7 has been responsible for several outbreaks and numerous sporadic cases of hemorrhagic colitis in the United States, Canada, and elsewhere (11). It has been strongly implicated as a causative agent in hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (11). When inoculated with O157:H7 EHEC, gnotobiotic piglets develop a watery diarrhea (5, 23). Bacteria diffusely colonize the cecal and colonic mucosa, attaching to enterocytes and resulting in microvillus effacement (5, 23). Enterocytes are affected to various degrees and may undergo degeneration and necrosis with desquamation from the mucosa. The colonic mesentery becomes markedly edematous, and suppurative inflammation occurs in the mucosa and submucosa (5, 23).

Several putative virulence factors are produced by EHEC, including toxins known as Shiga-like (or Vero) toxins I and II (SLT-I and SLT-II). Toxin-containing extracts from the culture supernatant of O157:H7 (EHEC) caused diarrhea in rabbits when given intragastrically, and histologic changes, including apoptosis, were observed in the intestinal epithelium (17). Apoptotic changes were also seen in ileal loops of adult rabbits inoculated with SLT-I (9). However, when gnotobiotic piglets were inoculated with an O157:H7 strain that had spontaneously lost its ability to produce SLT-I and -II, the same kind of intestinal lesion developed as in piglets inoculated with the SLT-I- and -II-producing parent strain (22). In addition, inoculation of gnotobiotic piglets with a non-verotoxigenic O101:K<sup>-</sup> *E. coli* strain resulted in attaching-effacing lesions (7). Thus, SLT-I and -II appear unessential for the production of diarrhea and attaching-effacing intestinal lesions in gnotobiotic piglets. It remains unclear whether the toxins contribute to hemorrhagic colitis in humans.

It has been suggested that the SLTs are of direct etiological importance in HUS and TTP (14) and that toxemia is the primary pathogenetic event, resulting in microvascular thrombi. The thrombi are hypothesized to result from a cytotoxic or cytopathic effect of SLT on vascular endothelium or induction of platelet aggregation (19).

This report describes clinical signs and lesions of the central nervous system (CNS) of gnotobiotic pigs infected with certain O157:H7 *E. coli* strains and presents evidence that SLT-II is involved in the pathogenesis. Some features of this disease of pigs may be similar to HUS or TTP in humans.

All *E. coli* strains used in the study except a nonpathogenic control strain were O157:H7. Salient characteristics of these strains are shown in Table 1. Tests for ability of strains to produce SLT-I and SLT-II were performed by Alison O'Brien (Uniformed Services University of the Health Sciences, Bethesda, Md.) as described elsewhere (16). Cultures for animal inoculation were grown overnight in 5 ml of brain heart infusion broth (approximately  $5 \times 10^9$  CFU/ml).

Piglets derived by closed hysterotomy and maintained in germfree isolators (1) were inoculated with bacterial test cultures per os at 1 day of age and observed a minimum of three times daily for signs of disease, including diarrhea, anorexia, depression, circling, loss of equilibrium, convulsions, and death. Animals that became depressed or exhibited signs of CNS disease were immediately euthanatized and necropsied. The rest of the animals were killed from 2 to 9 days postinoculation, usually at the rate of 1 piglet per group per day.

At necropsy, piglets were examined for gross pathological changes and tissue specimens were collected for histologic examination (brain and samples of liver, lung, spleen, kidney, jejunum, ileum, cecum, spiral colon, and rectum) and bacteriological culture (colon). Brains were sectioned in five areas (anatomic reference points), including medulla oblongata (olivary nucleus), cerebellum with medulla oblongata (cerebellar peduncles), midbrain (corpora quadrigemina), and two levels of cerebrum (interthalamic adhesion and genu of corpus callosum). Specimens for histologic examination were Formalin fixed and processed by standard methods. Sections of paraffin-embedded tissue were hematoxylin-eosin stained for light microscopy. Colon specimens were cultured aerobically on 5% sheep blood agar and Tergitol-7 agar and anaerobically on blood agar to assess the purity of the infection. The *E. coli* isolates were serogrouped to establish that they were the same as the inoculum.

Following challenge, all piglets, except one which was inoculated with O157:H7 strains of *E. coli*, developed diarrhea. The one nondiarrheic piglet exhibited signs of CNS disease at 2 days postinoculation. Signs of CNS disease, including imbalance, head tilt, or lateral recumbency, specific to one side, were observed 2 to 8 days postinoculation in 4 of 9 piglets inoculated with 933 and 3 of 6 inoculated with B2387. In addition, some piglets in both of these groups were depressed but did not exhibit signs of CNS disease. None of the piglets inoculated with 933D exhibited signs of CNS disease, nor were they depressed. Piglets inoculated with G58-1 remained clinically normal after challenge. Pure cultures of the *E. coli* strains used in inoculation were isolated

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TABLE 1. Characteristics of *E. coli* strains used in piglet challenge

Strain	Source (reference)	Serotype	SLT-I <sup>a</sup>	SLT-II <sup>a</sup>
933	Hamburger isolate (18)	O157:H7	+	+
933D	Derived from 933 <sup>b,c</sup> (21)	O157:H7	+	—
B2387	Human isolate <sup>c</sup>	O157:H7	—	+
G58-1	Swine isolate (5)	O101:K28:NM	—	—

<sup>a</sup> Detectable levels of toxins in culture supernatant (+, positive; —, negative).

<sup>b</sup> Spontaneously lost ability to produce SLT-II during storage.

<sup>c</sup> Obtained from A. D. O'Brien, Uniformed Services University of the Health Sciences, Bethesda, Md.

from the colon of each pig. All pigs inoculated with 933, 933D, and B2387 had mesocolonic edema. No other gross lesions were seen in these pigs or in the pigs inoculated with G58-1.

The cecal and colonic mucosa of pigs inoculated with 933, 933D, and B2387 were colonized by bacteria showing close adherence to the apical surface of enterocytes. Bacterial adherence was associated with enterocyte necrosis and desquamation, serosuppurative inflammation of the cecal and colonic submucosa, and edema of the mesocolon. The ilea of these pigs were occasionally colonized by closely adherent bacteria. Close bacterial adherence was not seen in the intestines of pigs inoculated with G58-1.

The brain stem, midbrain, and cerebrum of pigs inoculated with 933 and B2387, but not 933D or G58-1, frequently

TABLE 2. Incidence and distribution of malacia in the brains of pigs challenged with *E. coli* strains

Brain region (reference point)	No. of pigs with lesions/no. inoculated and examined for strain:			
	933	933D	B2387	G58-1
Medulla oblongata (olivary nucleus)	5/8	0/5	2/4	0/2
Cerebellum (cerebellar peduncles)	4/9	0/5	0/6	0/3
Midbrain (corpora quadrigemina)	4/9	0/5	3/6	0/4
Cerebrum (interthalamic adhesion)	2/9	0/5	6/6	0/4
Cerebrum (genu of corpus callosum)	6/9	0/5	6/6	0/4
Total with malacia/total inoculated	8/9	0/5	6/6	0/4

contained areas of arteriolar necrosis and malacia (Fig. 1; Table 2). Malacia was often bilateral, involved both grey and white matter, and in the cerebrum was primarily limited to subcortical areas, often in close proximity to basal nuclei. Focal areas of parenchymal necrosis associated with ringlike hemorrhages and arteriolar necrosis were occasionally seen in the cerebellum, the medulla oblongata, and the cerebrum (Fig. 2). Small hemorrhages associated with platelet and fibrinous capillary thrombi were also occasionally seen in all levels of the brain. Arterioles in the brain were frequently affected with endothelial swelling, necrosis and regeneration, and necrosis of myocytes in the tunica media (Fig. 3). Lesions were not seen in other tissues examined.

Lesions in the brains of piglets in this study appeared to

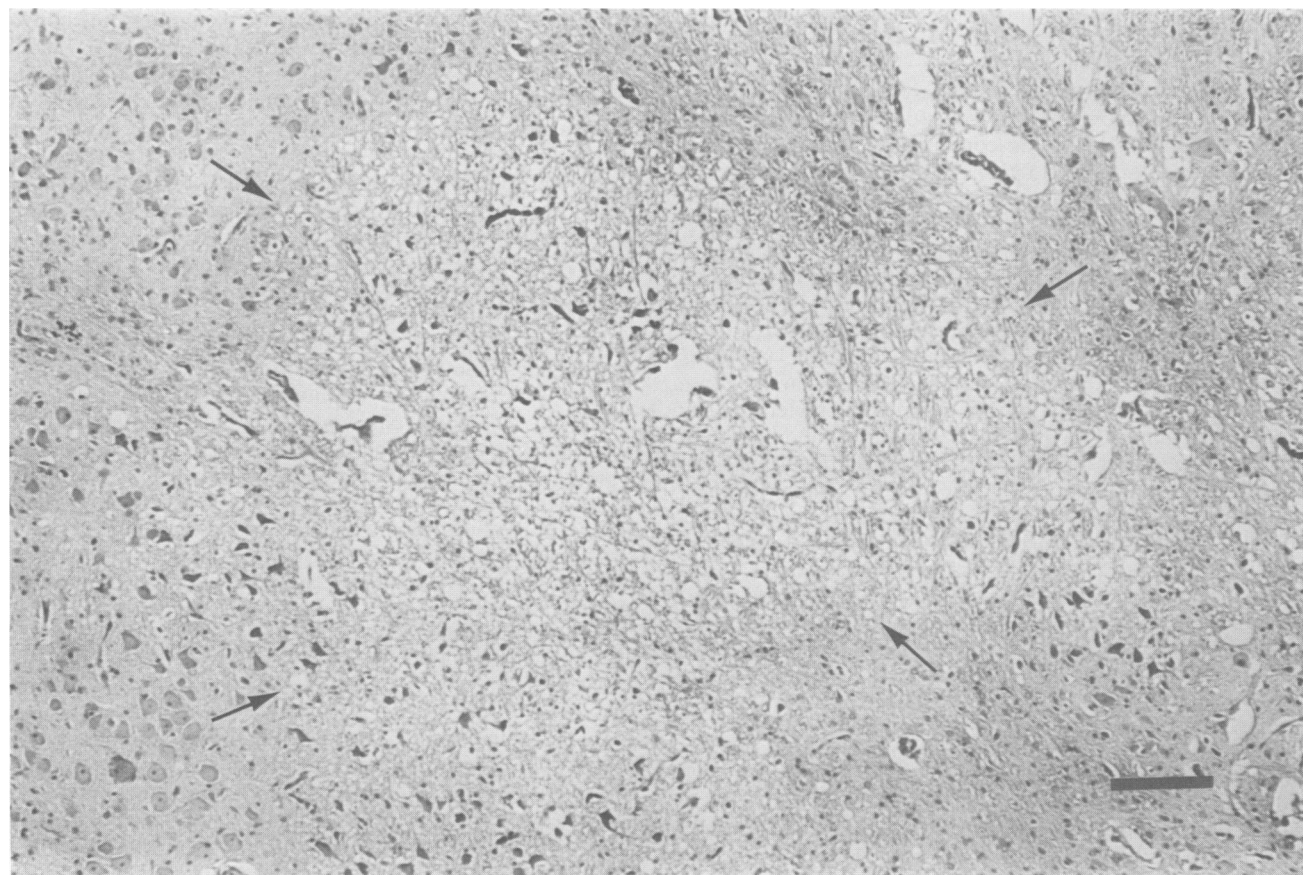


FIG. 1. Acute focal malacia (center, boundaries demarcated by arrows) in ventral medulla oblongata of piglet 4.5 days postinoculation with 933. Necrosis is indicated by nuclear pyknosis, cytoplasmic eosinophilia of neurons and glial cells, and vacuolation of neuropil. Bar, 200  $\mu$ m.

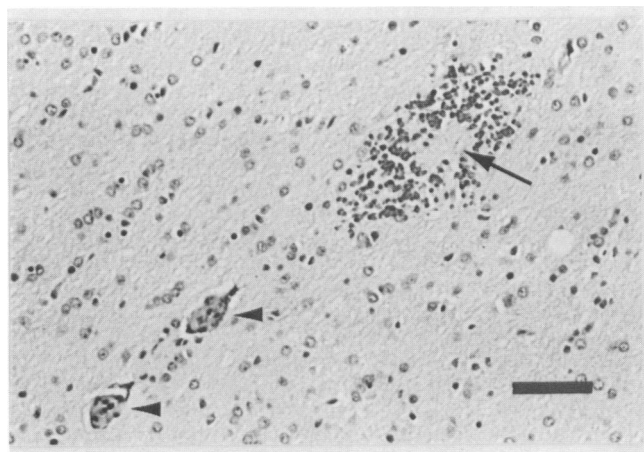


FIG. 2. Focal necrosis (arrow) in cerebellar peduncle of piglet (see legend to Fig. 1). Necrotic focus is demarcated by a ring of hemorrhage and is associated with arteriolar necrosis (arrowheads). Bar, 100  $\mu$ m.

result from a toxemia associated with the production of SLT-II by bacteria in the intestinal lumen. Only piglets inoculated with O157:H7 strains that produced SLT-II developed identifiable brain lesions. Loss of genes coding for SLT-II production in strain 933 was apparently accompanied by loss of ability to cause brain lesions.

The lesions in the brains of pigs infected with SLT-II-producing *E. coli* were essentially identical to lesions resulting from natural infection of pigs with edema disease-producing strains of *E. coli* (10). The same lesions have been produced by intravenous injection of a partially purified preparation of toxin (edema disease principle) from edema disease-producing strains (2). Edema disease-causing strains produce a substance (presumably edema disease principle) that is cytotoxic to Vero cells (3, 20) and neutralizable by antiserum to SLT-II (12). Furthermore, the genes for this substance (known as SLT-II variant) are highly homologous (94% for subunit A genes and 79% for subunit B genes) (24). Similarities in the two toxins may account for similarities in the nature of the lesions produced in the brains of pigs and

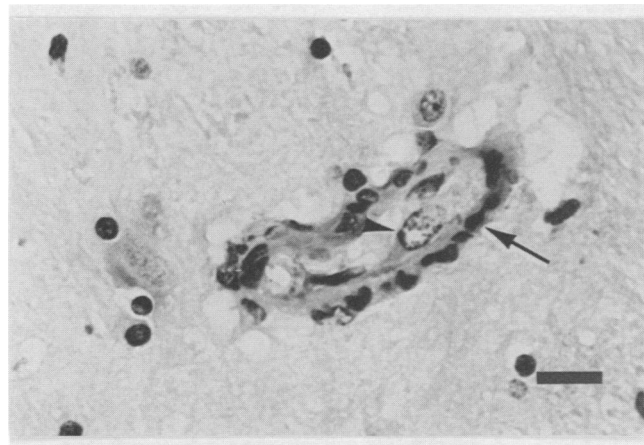


FIG. 3. Arteriole in cerebellar peduncle of piglet (see legend to Fig. 1). Endothelial cell appears regenerative with large, vesicular nucleus and basophilic cytoplasm (arrowhead). Myocytes in tunica media are necrotic (arrow). Bar, 25  $\mu$ m.

the anatomic locations of these lesions. Differences in the site of edema (mesocolon) in our experimental pigs, when compared with those most frequently found in natural edema disease, may have been the result of one of the following: (i) differences in bacterial colonization sites in the two diseases, (ii) differences in the effect of toxin on newborn versus older pigs, or (iii) differences in cell surface binding by the two toxins. Differences between SLT-II and SLT-IIv in receptor activity are suggested by the observation that SLT-II is cytotoxic to both Vero and HeLa cells, whereas SLT-IIv is cytotoxic to Vero cells only (12).

Edema disease of pigs and HUS and TTP of humans have a number of features in common. Both the pig and human diseases frequently have a prodrome of diarrhea (8, 14, 15) and may include submucosal colonic edema (14, 15). The CNS may be affected in any of these diseases (6, 8, 10, 14, 15). Each of them involves the vascular system (4, 6, 14, 15), and endothelial cell damage may be the primary event that precipitates clinical manifestations of the disease (14). Endothelial swelling was seen following experimental infection of swine with an edema disease-producing strain of *E. coli* (13) and is observed in the glomeruli of patients with HUS (4).

Glomerular damage, as found in HUS and apparently resulting from fibrinous thrombosis (4), was not observed in the piglets infected with O157:H7 *E. coli*, nor is it observed in association with edema disease. This suggests that some of the lesions associated with infection by O157:H7 *E. coli* are host specific. Perhaps only endothelial cells in certain tissues, varying somewhat from species to species, are susceptible to damage by SLT-II. Susceptibility may be the result of expression of cell surface receptors to that toxin.

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